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(54) HOEFCC11, a HAS2 splicing variant

(57) HOEFC 11 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing HOEFC 11 polypeptides and polynucleotides in the design of protocols for the treatment of chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease, among others, and diagnostic assays for such conditions.

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Description

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to the hyaluronan synthase family, hereinafter referred to as HOEFC11. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides

BACKGROUND OF THE INVENTION

Hyaluronic acid (HA), an important constituent of extracellular matrix, is a linear polysaccharide of alternating glucuronic acid and N-acetyl glucosamine residues. It is synthesized by a membrane-bound enzyme hyaluronan synthase (HAS) and extruded into the extracellular space. Cloning of two human HAS (HAS 1 and HAS 2) has been reported very recently (K. Watanabe and Y. Yamaguchi, J. Biol. Chem. 271:22945-22948, 1996) (N. Itano and K. Kimata, Biochem. Biophy. Res. Communications, 222:816-820, 1996). HA synthesis is involved in many cellular functions such as migration, invasion, adhesion, transformation, proliferation and wound healing. HA synthesis has been shown to be induced by FBS, PDGF, EGF, IL-1, retinoic acid, IGF, TGF beta, etc. Increased HA production is: (a) a general phenomenon in various organs attacked by inflammatory cells, (b) implicated in tissue edema, (c) a characteristic of tissue remodeling and (d) a marker for early stage of extracellular matrix remodeling following vascular injury Increased levels of HA have been reported in chronic renal failure, inflammatory, diseases, cancer (prostate, mammary and orther invasive tumors), aortas fi-om diabetic patients, smaller airways of patients with acute alveolitis, transplantation edema in rejecting heart and kidney, myocardial ischemia, balloon injury, liver cirrhosis, wound healing and angiogenesis. Hyaluronidase (breaks down HA) is reported to be beneficial in limiting cellular damage during myocardial ischemia in rat, dog and man. This indicates that the hyaluronan synthase family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of the hyaluronan synthase family which can play a role in preventing, ameliorating or connecting dysfunctions or diseases, including, but not limited to, chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to HOEFC11 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such HOEFC11 polypeptides and polynucleotides. Such uses include the treatment of chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with HOEFCI 1 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HOEFC11 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"HOEFC11" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"HOEFC11 activity or HOEFC11 polypeptide activity" or "biological activity of the HOEFC11 or HOEFC11 polypeptide" refers to the metabolic or physiologic function of said HOEFC11 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said HOEFC11.

"HOEFC11 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide sep-

arated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide

sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al., Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al., J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to HOEFC11 polypeptides. The HOEFC11 polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within HOEFC11 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably HOEFC11 polypeptide exhibit at least one biological activity of HOEFC11.

The HOEFC11 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the HOEFC11 polypeptides are also included in the invention. A fragment is a polypeptide having an ammo acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned HOEFC11 polypeptides. As with HOEFC11 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of HOEFC11, polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of HOEFC11 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments.

Biologically active fragments are those that mediate HOEFC11 activity, including those with a similar activity or an improved activity or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the HOEFC11, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The HOEFC11 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to HOEFC11 polynucleotides. HOEFC11 polynucleotides include isolated polynucleotides which encode the HOEFC11 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, HOEFC11 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 encoding a HOEFC11 polypeptide of SEQ ID NO: 2, and polynucleotide having the particular sequence of SEQ ID NO:1. HOEFC11 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the HOEFC11 polypeptide of SEQ ID NO:2 over its entire length, and a polynucleotide that is at least 80% identical to that having SEQ ID NO: 1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under HOEFC11 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such HOEFC11 polynucleotides.

HOEFC11 of the invention is structurally related to other proteins of the hyaluronan synthase family, as shown by the results of sequencing the cDNA of Table 1 (SEQ ID NO: 1) encoding human HOEFC11. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 152 to 974) encoding a polypeptide of 241 amino acids of SEQ ID NO:2. The amino acid sequence of Table 2 (SEQ ID NO:2) has about 99.5% identity (using FASTA) in 210 amino acid residues with hyaluronan synthase (HAS2) (K. Watanabe and Y. Yamaguchi, J. Biol. Chem. 271: 22945-22948, 1996). Most importantly, HOEFC11 is a naturally occurring truncation of the HAS2, missing 342 amino acids at the carboxyl terminus. The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 99.7% identity (using FASTA) in 877 nucleotide residues with hyaluronan synthase (HAS2)(K.. Watanabe and Y. Yamaguchi, J. Biol. Chem. 271:22945-22948). Most importantly, HOEFC11 is a naturally occuring truncation of HAS2, missing 1026 bp at the 3' end of the coding region.

Table 1[a]

GCACGAGCTGAAGTGCAACGGAAACATAAAGAGAATATTA	40
GTGAAATTATTTTTTAAAGTGGGGAAgAATCAAACATTTA	80
AgACTCCCCTATCCTTTTTAAATGTTGTTTTTAAATTTCT	120
TATTTTTTTTGGCCGGTCGTCTCAAATTCATCTGATCTCT	160
TATTACCTCAATTTTGGAAACTGCCCGCCACCGACCCTCC	200
GGGACCACACAGACaGGCTGAGGACgACTTTATGACCAAG	240
AGCTGAACAAGATGCATTGTGAGAGGTTTCTATGTATCCT	280
GAGAATAATTGGAACCACACTCTTTGGAGTCTCTCTCCTC	320
CTTGGAATCaCAGCTGCTTATATTGTTGGCTACCAGTTTA	360
TCCAAACGGATAATTACTATTTCTCTTTTGGACTGTATGG	400
TGCCTTTTTGGCATCACACCTCATCATCCAAAGCCTGTTT	440
GCCTTTTTGGAGCACCGAAAAATGAAAAAATCCCTAGAAA	480
CCCCCATAAAGTTGAACAAAACAGTTGCCCTTTGCATCGC	520
TGCCTATCAAGAAGATCCAGACTACTTAAGGAAATGTTTG	560
CAATCTGTGAAAAGGCTAACCTACCCTGGGATTAAAGTTG	600
TCATGGTCATAGATGGGAACTCAGAAGATGACCTTTACAT	640
GAtGGACATCTTCAGTGAAGTCATGGGCAGAGACAAATCA	680
GCCACTCATATcTGGAAGAACAACTTCCACGAAAAGGGTC	720
CCGGTGAGACAGATGAGTCACATAAAGAAAGCTCGCAACA	760
CGTAACGCAATTGGTCTTGTCCACAAAAGTATcTGCATC	800
ATGCAAAAATGGGGTGGAAAAAGAGAAGTCATGTACACAG	840
CCTTCAGAGCACTGGGACGAAGTGTGGATTATGTACAGGT	880
AGGTCTCCACATTCCTGCCAGGGCAAACATACATTTAAAT	920
AAAGCCGCTTTTGTATCTGTCCAGTCATATGCTATAGCCC	960
ATCCTTGTCCCTTCTGAACACAGTACTTCTTTCAGTTCAT	1000
TTGAAAACAGCATGACTGTTGAAAGCACATTTTGAAAAAA	1040
AAAAAAAAAA	1051

[a] A nucleotide sequence of a human HOEFC11 (SEQ ID NO: 1).

Table 2[b]

MHCERFLCILRIIGTTLFGVSLLLGITAAYIVGYQFIQTD	40
NYYFSFGLYGAFLASHLIIQSLFAFLEHRKMKKSLETPIK	80
LNKTVALCIAAYQEDPDYLRKCLQSVKRLTYPGIKVVMVI	120
DGNSEDDLYMMDIFSEVMGRDKSATHIWKNNFHEKGPGET	160
DESHKESSQHVTQLVLSNKSICIMQKWGGKREVMYTAFRA	200
LGRSVDYVQVGLHIPARANIHLNKAAEVSVQSYATAHPCP	240
F	241

[b] An amino acid sequence of a human HOEFC11 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding HOEFC11 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human osteoblasts using the expressed sequence tag (EST) analysis (Adams, M.D., *et al, Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature,* (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995)377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding HOEFC11 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 152 to 974 of SEQ ID NO: 1). or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of HOEFC11 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding HOEFC11 variants comprise the amino acid sequence HOEFC11 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding HOEFC11 polypeptide and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the HOEFC11 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding HOEFC11 polypeptide comprises the steps of screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof. and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, HOEFC11 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof. Also included with HOEFC11 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured,

sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL* (supra).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the HOEFC11 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If HOEFC11 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

HOEFC11 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of HOEFC11 polynucleotides for use as diagnostic reagents. Detection of a mutated form of HOEFC11 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of HOEFC11. Individuals carrying mutations in the HOEFC11 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HOEFC11 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting

temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al., Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al., Proc Natl Acad Sci USA* (1985) 85:4397-4401.

In another embodiment, an array of oligonucleotides probes comprising HOEFC11 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease through detection of mutation in the HOEFC11 gene by the methods described.

In addition, chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of HOEFC11 polypeptide or HOEFC11 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an HOEFC11 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the HOEFC11 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the HOEFC11 polypeptides can be obtained by administering the polypeptides or epitope-beanng fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al., Immunology Today* (1983)4:72) and the EBV-hybridoma technique (Cole *et al.,* MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against HOEFC11 polypeptides may also be employed to treat chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with HOEFC11 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering HOEFC11 polypeptide via a vector directing expression of HOEFC11 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a HOEFC11 polypeptide wherein the composition comprises a HOEFC11, polypeptide or HOEFC11 gene. The vaccine formulation may further comprise a suitable carrier. Since HOEFC11 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The HOEFC11 polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the HOEFC11 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al., Current Protocols in Immunology* 1(2):Chapter 5(1991).

HOEFC11 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate HOEFC11 polypeptide on the one hand and which can inhibit the function of HOEFC11 1 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease.

In general, such screening procedures may involve using appropriate cells which express the HOEFC11 polypeptide or respond to HOEFC11 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the HOEFC11 polypeptide (or cell membrane containing the expressed polypeptide) or respond to HOEFC11 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for HOEFC11 activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the HOEFC11 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the HOEFC11 polypeptide, using detection systems appropriate to the cells bearing the HOEFCI polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

The HOEFC11 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of HOEFC11 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of HOEFC11 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of HOEFC11 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The HOEFC11 protein may be used to identify membrane bound or soluble receptors, if any, through standard

receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the HOEFC11 is labeled with a radioactive isotope (eg 125I), chemically modified (eg biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of HOEFC11 which compete with the binding of HOEFC11 to its receptors. Standard methods for conducting screening assays are well understood in the art.

Examples of potential HOEFC11 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, etc., as the case may be, of the HOEFC11. 1 polypeptide, e.g., a fragment of the ligands, substrates, receptors, or small molecules which bind to the polypetide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic renal failure, inflammatory diseases, myocardial ischemia, cancer, rheumatoid arthritis, cirrhotic liver disease, related to both an excess of and insufficient amounts of HOEFC11 polypeptide activity.

If the activity of HOEFC11 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the HOEFC11 polypeptide, such as, for example, by blocking the binding of ligands, substrates, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of HOEFCI 1 polypeptides still capable of binding the ligand. substrate, etc. in competition with endogenous HOEFC11 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the HOEFC11 polypeptide.

In another approach, soluble forms of HOEFC11 polypeptides still capable of binding the ligand in competition with endogenous HOEFC11 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the HOEFC11 polypeptide.

In still another approach, expression of the gene encoding endogenous HOEFC11 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991)56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979) 6:3073; Cooney *et al., Science* (1988) 241:456; Dervan *et al, Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of HOEFC11 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates HOEFC11 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of HOEFC11 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches,* (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of HOEFC11 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of HOEFC11 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other

compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

HAS 2 has 6 predicted potential transmembrane domains, 2 in the N-terminal and 4 in the C-terminal regions (K. Watanabe and Y. Yamaguchi, J. Biol. Chem. 271:22945-22948, 1996). In the middle of the polypeptide, there are 5 amino acid residues that are thought to be crucial for the N-acetylglucosaminyltransferase activity in the Streptococcus HA synthase (S. Nagahashi, et at., J. Biol. Chem. 270:13961-13967, 1995). The synthesis of HA increases in proliferating fibroblasts while it is inhibited in growth-arrested cells (M. Brecht, et al., Biochem. J. 239:445-450, 1986; K. Matuoka, et al. J. cell Biol. 104 1105-1115, 1987. J. R. Kitchen, et al , Biolchem. J. 309:649-656, 1995) However, little is known about the regulation of HA synthesis. Here, we identified a novel splicing variant of HAS2, HOEFC11, which missed the 5 crucial amino acids for the enzyme activity and the 4 transmembrane domains in the C-terminus. This variant form of HAS2 may play a regulatory role in the HA synthesis by acting as a dominant negative inhibitor of HAS2 enzyme. This mechanism has been well demonstrated in the study of aldehyde dehydrogenase, ornithine transcarboxylase, as well as many membrane-bound receptors (Y. Nakamura and H. Nakauchi, Sci. 264:588-589; R. Ebner, et al., Sci. 260:1344-1348; S. Werner, et al., EMBO. J. 12:1635-2643).

A search of a random cDNA sequence database from Human Genome Sciences consisting of short sequences known as expressed sequence tags (ESTs) using BLAST algorithm disclosed an EST (# 1750866)which was homologous to human hyaluronan synthase (HAS2). FIGS EST 1750866 has the following sequence:

```
  1  CTGAAGTGCA AGNAAACATA AAGAGAATAT TAGTGAAATT ATTTTTTAAA

 51  GTGGGGAAGA ATCAAACATT TAAGACTCCC CTATCCTTTT TAAATGTTGT

101  TTTTAAATTT CTTATTTTTT TTGGCCGGTC GTCTCAAATT CATCTGATCT

151  CTTATTACCT CAATTTTGGA AACTGCCCGC CACCGACCCT CCGGGGACCA

201  CACAGACAGG CTGAGGACGA CTTTATGACC AAGAGCTGAA CAAGAGNCAT

251  TGTGAGAGGT TCCAAGGAAC CNGNAGATAA TTGGGANCCA AACCTTTGGN

301  GGT  (SEQ ID NO:3)
```

In order to obtain the full length clones, a complete DNA sequence of the inserts were deduced using automated

DNA sequencing procedure. One of the clones, HOEFC11, contained a 1 kb insert. A map analysis of the DNA sequence using the Lasergene software indicated an open reading frame (ORF) which was a truncated form of HAS2. In order to confirm the identity of the clone, PCR primers were designed using the nucleotide sequence of the open reading frame (ORF). A DNA fragment with the correct size was amplified from human prostate and placenta mRNA and subcloned into pCR2.1 vector from Invitrogen (San Diego, CA). The DNA sequence was identical to the open reading frame (ORF) of HOEFC11.

Annex to the description

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: NOVEL HAS2 SPLICING VARIANT HOEFC11: A TARGET IN CHRONIC RENAL FAILURE, INFLAMMATORY DISEASES AND MYOCARDIAL ISCHEMIA

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCR ADDRESS:
(A) ADDRESSSEE: SmithKline Beecham, Corporate Tnrellectual Property
(B) STREET: Two New Horizons Court
(C) CITY: Brentford
(D) STATE: Middlesex
(E) COUNTRY: United Kingdom
(F) ZIP: TW8 9EP

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 29-MAY-1997
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: CONNELL, Anthony Christopher
(B) REGISTRATION NUMBER: 5630

(C) REFERENCE/DOCKET NUMBER: GH-70053

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +44 1279 644 395
(B) TELEFAX: +44 181 975 6294
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1051 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCACGAGCTG AAGTGCAACG GAAACATAAA GAGAATATTA GTGAAATTAT TTTTTAAAGT 60
GGGGAAGAAT CAAACATTTA AGACTCCCCT ATCCTTTTTA AATGTTGTTT TTAAATTTCT 120
TATTTTTTTT GGCCGGTCGT CTCAAATTCA TCTGATCTCT TATTACCTCA ATTTTGGAAA 180
CTGCCCGCCA CCGACCCTCC GGGACCACAC AGACAGGCTG AGGACGACTT TATGACCAAG 240
AGCTGAACAA GATGCATTGT GAGAGGTTTC TATGTATCCT GAGAATAATT GGAACCACAC 300
TCTTTGGAGT CTCTCTCCTC CTTGGAATCA CAGCTGCTTA TATTGTTGGC TACCAGTTTA 360
TCCAAACGGA TAATTACTAT TTCTCTTTTG GACTGTATGG TGCCTTTTTG GCATCACACC 420
TCATCATCCA AAGCCTGTTT GCCTTTTTGG AGCACCGAAA AATGAAAAAA TCCCTAGAAA 480
CCCCCATAAA GTTGAACAAA ACAGTTGCCC TTTGCATCGC TGCCTATCAA GAACATCCAG 540
ACTACTTAAG GAAATGTTTG CAATCTGTGA AAAGGCTAAC CTACCCTGGG ATTAAAGTTG 600
TCATGGTCAT AGATGGGAAC TCAGAAGATG ACCTTTACAT GATGGACATC TTCAGTGAAG 660
TCATGGGCAG AGACAAATCA GCCACTCATA TCTGGAAGAA CAACTTCCAC GAAAAGGGTC 720
CCGGTGAGAC AGATGAGTCA CATAAAGAAA GCTCGCAACA CGTAACGCAA TTGGTCTTGT 780
CCAACAAAAG TATCTGCATC ATGCAAAAAT GGGGTGGAAA AAGAGAAGTC ATGTACACAG 840
CCTTCAGAGC ACTGGGACGA AGTGTGGATT ATGTACAGGT AGGTCTCCAC ATTCCTGCCA 900
GGGCAAACAT ACATTTAAAT AAAGCCGCTT TTGTATCTGT CCAGTCATAT GCTATAGCCC 960
ATCCTTGTCC CTTCTGAACA CAGTACTTCT TTCAGTTCAT TTGAAAACAG CATGACTGTT 1020
GAAAGCACAT TTTGAAAAAA AAAAAAAAA A 1051

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met His Cys Glu Arg Phe Leu Cys Ile Leu Arg Ile Ile Gly Thr Thr
 1               5                  10                  15
Leu Phe Gly Val Ser Leu Leu Leu Gly Ile Thr Ala Ala Tyr Ile Val
            20                  25                  30
Gly Tyr Gln Phe Ile Gln Thr Asp Asn Tyr Tyr Phe Ser Phe Gly Leu
        35                  40                  45
Tyr Gly Ala Phe Leu Ala Ser His Leu Ile Ile Gln Ser Leu Phe Ala
    50                  55                  60
Phe Leu Glu His Arg Lys Met Lys Lys Ser Leu Glu Thr Pro Ile Lys
65                  70                  75                  80
Leu Asn Lys Thr Val Ala Leu Cys Ile Ala Ala Tyr Gln Glu Asp Pro
                85                  90                  95
Asp Tyr Leu Arg Lys Cys Leu Gln Ser Val Lys Arg Leu Thr Tyr Pro
            100                 105                 110
Gly Ile Lys Val Val Met Val Ile Asp Gly Asn Ser Glu Asp Asp Leu
        115                 120                 125
Tyr Met Met Asp Ile Phe Ser Glu Val Met Gly Arg Asp Lys Ser Ala
    130                 135                 140
Thr His Ile Trp Lys Asn Asn Phe His Glu Lys Gly Pro Gly Glu Thr
145                 150                 155                 160
Asp Glu Ser His Lys Glu Ser Ser Gln His Val Thr Gln Leu Val Leu
                165                 170                 175
Ser Asn Lys Ser Ile Cys Ile Met Gln Lys Trp Gly Gly Lys Arg Glu
            180                 185                 190
Val Met Tyr Thr Ala Phe Arg Ala Leu Gly Arg Ser Val Asp Tyr Val
        195                 200                 205
Gln Val Gly Leu His Ile Pro Ala Arg Ala Asn Ile His Leu Asn Lys
    210                 215                 220
Ala Ala Phe Val Ser Val Gln Ser Tyr Ala Ile Ala His Pro Cys Pro
225                 230                 235                 240
Phe
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 303 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CTGAAGTGCA AGNAAACATA AAGAGAATAT TAGTGAAATT ATTTTTTAAA GTGGGGAAGA   60
ATCAAACATT TAAGACTCCC CTATCCTTTT TAAATGTTGT TTTTAAATTT CTTATTTTTT  120
TTGGCCGGTC GTCTCAAATT CATCTGATCT CTTATTACCT CAATTTTGGA AACTGCCCGC  180
CACCGACCCT CCGGGGACCA CACAGACAGG CTGAGGACGA CTTTATGACC AAGAGCTGAA  240
CAAGAGNCAT TGTGAGAGGT TCCAAGGAAC CNGNAGATAA TTGGGANCCA AACCTTTGGN  300
GGT                                                                303
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Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the HOEFC11 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said polynucleotide.

2. The polynucleotide of claim 1 which is DNA or RNA.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that has at least 80% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 wherein said nucleotide sequence comprises the HOEFC11 polypeptide encoding sequence contained in SEQ ID NO:1.

5. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a HOEFC11 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a HOEFC11 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a HOEFC11 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a HOEFC11 polypeptide.

10. A HOEFC11 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the HOEFC11 polypeptide of claim 10

13. A method for the treatment of a subject in need of enhanced activity or expression of HOEFC11 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the HOEFC11 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of subject having need to inhibit activity or expression of HOEFC11 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate , or receptor.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of HOEFC11 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said HOEFC11 1 polypeptide in the genome of said subject, and/or
(b) analyzing for the presence or amount of the HOEFC11 polypeptide expression in a sample derived from said subject.

16. A method for identifying compounds which inhibit (antagonize) or agonize the HOEFC11 polypeptide of claim 10 which comprises:

(a) contacting a candidate compound with cells which express the HOEFC11 polypeptide (or cell membrane expressing HOEFC11 polypeptide) or respond to HOEFC11 polypeptide; and
(b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for HOEFC11 polypeptide activity.

17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.